FURTHER STUDIES ON THE PARTHENOGENETIC DEVELOPMENT OF THE STARFISH EGG.

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The results of investigations described in an earlier paper[1] led me to the view that possibly a conjugation of egg and sperm chromosomes, similar to that apparently occurring in starfish eggs that had been treated with CO_2 and subsequently fertilized, might be found to occur in normally fertilized eggs.

As I suggested in the paper mentioned, it would be necessary, in order to settle the question raised, to reëxamine the normal fertilization and cleavage stages or to make a study of the formation of the germ cells in the starfish.

This paper deals with observations made in accordance with this plan and with some further observations made on starfish eggs. developing as a result of treatment with CO_2. The material for the investigation was obtained while I was occupying a room at the Marine Biological Laboratory, Woods Hole, during a portion of the summer of 1906.

I found, soon after beginning a study of fertilized starfish eggs. that the equatorial plate of the first cleavage spindle contained, with variations which I shall mention later, in eggs from some individuals 18 chromosomes, and in eggs from other individuals 36 chomosomes. I have as yet been unable to correlate this difference in the number of chromosomes with the common starfishes of the Woods Hole region, *Asterias forbesii* and *Asterias vulgaris*,[2] although it is probable that such a relationship will be established.

The study of the fertilized eggs proved puzzling, and it was not until I had made an investigation of the spermatogenesis of *Asterias vulgaris* and a reëxamination of eggs developing parthenogenetically after treatment with CO_2 that I was able to find a solution for the problem under consideration.

[1] "Studies on the Development of the Starfish Egg," D. H. Tennent and M. J. Hogue, *Journal of Experimental Zoölogy*, Vol. III. (1906).

[2] Clark, "The Echinoderms of the Woods Hole Region," Bull. U. S. F. C., Vol. XXII. (1902), pp. 553–554.

Inasmuch as the basis of my interpretation lies in facts observed during the study of the male germ cells, I shall first present a brief account of these observations.

The Spermatogenesis of Asterias vulgaris.

In well-preserved stronger Flemming material stained in iron-hæmatoxylin the spermatogonia show 18 chromosomes, these all having a slightly constricted or dumb-bell form (Fig. 1). The chromosomes are either straight or slightly bent.

The chromosomes of the primary spermatocytes are nine in number and have at first a distinct dumb-bell form. A precocious longitudinal splitting soon gives them a V or looped form which may be seen in horizontal sections of the equatorial plate (Fig. 2).

1 2 3 4 5

Fig. 1. Equatorial plate of spermatogonial mitosis.
Fig. 2. Spermatocyte of the 1st order.
Fig. 3. Spermatocyte of the 2d order. Polar view.
Fig. 4. Spermatocyte of the 2d order. Metaphase.
Fig. 5. Second spermatocyte division.

The secondary spermatocytes contain nine chromosomes (Figs. 3 and 4). In the second maturation mitosis these appear to be divided transversely (Fig. 5), giving nine as the reduced germ-cell number.

Studies on Eggs.

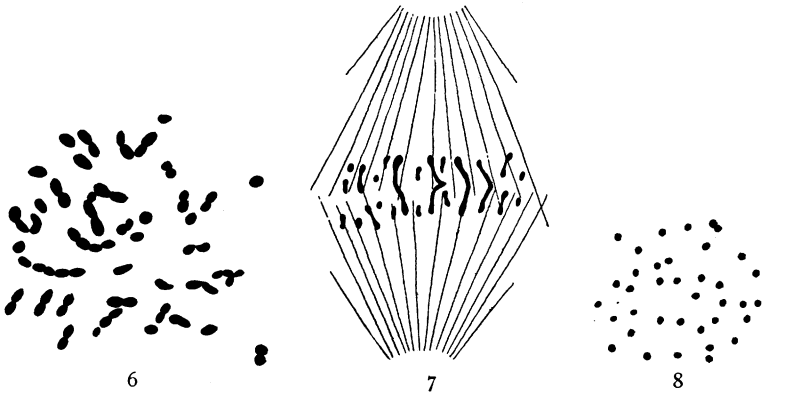
After noting the difference in the somatic number of chromosomes in the different lots of eggs sectioned, it became evident that it was desirable to have a set of material in which the eggs from one individual had been treated in three different ways, namely, one set fertilized with sperm, another set treated with CO_2 and a third set treated with CO_2 and subsequently fertilized. I succeeded in obtaining one lot of material of this nature. I shall record my observations in the order of the above statement.

(*a*) *Observations on Fertilized Eggs.*

In a successful preparation, the section being sufficiently thick to show the greater number of the chromosomes of the equa-

torial plate of the first segmentation spindle in their entirety (Fig. 6), the chromosomes are seen to be of a dumb-bell form, some straight, some slightly bent, and lying with their long axis placed transversely to the spindle fibers. Their number, as may be seen from the figure, would lead one to suspect 36 as the somatic number, but owing to the fact that some of the chromosomes are cut, this may not be stated with certainty.

In an especially fortunate section passing symmetrically through the long axis of the spindle, it is seen that the chromosomes have been split longitudinally and drawn out as somewhat slender rods. In drawing this figure I have shown only the chromosomes and parts of chromosomes lying within a short



6 7 8

FIG. 6. Equatorial plate 1st segmentation fertilized egg. Polar view.
FIG. 7. First segmentation fertilized egg. Anaphase.
FIG. 8. First segmentation fertilized egg. Section through chromosomes as they are drawn out in anaphase.

focal range, inasmuch as it would have complicated the figure so greatly as to make it unintelligible had the chromosomes lying toward the opposite side of the spindle been added. In this section again, the full number of chromosomes could not be counted with certainty.

Finally, in a section passing transversely through the spindle of an egg in the same stage of division as that from which Fig. 7 was drawn, it is shown conclusively that the somatic number of chromosomes in this lot of fertilized eggs is 36.

(*b*) *Observations on Eggs Treated with* CO_2.

Due precautions were of course taken to avoid chance fertilization. The control showed freedom from segmenting eggs.

In the sections of these eggs it was even more difficult than in the fertilized eggs to determine accurately the number of chromosomes. Sections thick enough to contain all of the chromosomes were unintelligible. Thinner sections were likewise of

FIG. 9. *a–b*. Sections through same equatorial plate CO_2 egg.

little value. Fig. 9 shows all of the chromatic material contained in the equatorial plate as demonstrated in two sections of this egg. The impossibility of stating with any reasonable degree of accuracy the number of chromosomes involved is evident

FIG. 10. *a–d*. Four longitudinal sections through 1st segmentation spindle, late anaphase. CO_2 egg. Spindle fibers omitted.

to any observer. Nor is the situation appreciably relieved by the examination of longitudinal sections of the spindle in later anaphase.

In such sections as those shown in Fig. 10, *a–d*, a cursory

examination would lead the observer to believe that the number of chromosomes is fully as great as that in the fertilized eggs. This view might be supported by the fact that many of the chromosomes show a form similar to that possessed by those of the fertilized eggs.

Closer examination of the position and arrangement of the chromosomes in these sections reveals the fact that the bodies have been sectioned. The imaginary superposition of one figure upon the other lends credence to such an idea.

For these reasons I have been unable to determine the number of chromosomes by actual count. The number I believe to be 18, a statement for which I shall give my reasons later.

(*c*) *Eggs Treated with CO_2 and Subsequently Fertilized.*

Sections of these eggs agree with figures that I have already published. The eggs were fertilized and underwent segmentation, the CO_2 simply retarding the rate of development.

Observations on Other Starfish Eggs Treated with CO_2.

I succeeded in obtaining one lot of eggs which developed after treatment with CO_2, that contain in all cases only 9 chromosomes. I was unable to obtain a ripe male at the time and so can give no facts as to the fertilization of these eggs.

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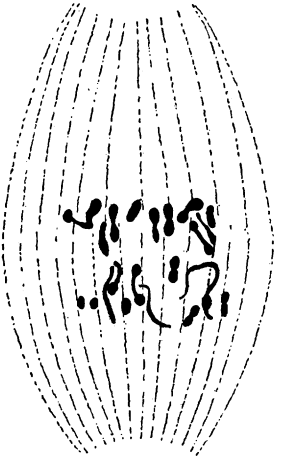

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Fig. 11. First segmentation CO_2 egg. Early anaphase.

Fig. 12. First segmentation CO_2 egg. Later anaphase.

The individual from which I obtained the eggs I identified as *Asterias forbesii* although it will be noted that the number of chromosomes agrees with the germ-cell number in my spermatogenesis material of *Asterias vulgaris*.

In these eggs, as in the other CO_2 eggs described, the form of the chromosomes is irregular but owing to the smaller number may be counted readily. The equatorial plate, the daughter plates, etc., all show the same number, — *i. e.*, nine (Figs. 11 and 12).

Fig. 13 shows the extremely irregular form assumed by the chromatic material in anaphase and explains the reasons for the complexity exhibited by sections such as those from which Fig. 10 was drawn.

13

14

FIG. 13. First segmentation CO_2 egg. Anaphase.

FIG. 14. First segmentation CO_2 egg. Late anaphase. One chromosomal vesicle.

As shown in Fig. 13 this chromatic material at this stage is in the form of greatly twisted threads. A single section may cut the thread in several places.

In later anaphase these threads are drawn out, passing through a variety of changes and at last are embodied in chromosomal vesicles which unite to form the daughter nucleus (Fig. 14).

Clearly then, this egg with its oöcyte number of 9 chromosomes does not exhibit the phenomenon of "autoregulation."

As I have already stated, owing to my inability to make an accurate count, I have not been able to show this to be true in the case of the egg whose reduced number of chromosomes is 18. That the behavior of both eggs is probably similar will be granted by most readers.

General Considerations.

These observations show conclusively that in the fertilized egg there is no conjugation of maternal and paternal chromosomes as individuals, at the time when I thought that such a union might take place.

The facts that I have given, namely, that the reduced number of chromosomes in the male germ cell of one form is 9, that the reduced number of chromosomes in one egg is 18, and that the number in fertilized eggs is 18 and 36 is sufficient proof.

After the examination of many lots of fertilized eggs I became convinced that 18 and 36 were not constant as somatic numbers. Small variations, such as differences of one or two, might be laid to error in counting. A constant greater variation that I have found hardly seems due to the same cause.

A possible interpretation of such a greater variation is of interest.

In one lot of eggs the number 27 seems constant. In this lot I have never been able to count as many as 36 chromosomes.

Such a number, (27), is readily explained on the supposition that an egg containing 18 chromosomes has been fertilized by a spermatozoan containing 9, or that an egg with 9 has been fertilized by a spermatozoan containing 18. The result in either case would be a somatic number of 27.

Now, accepting the interpretation of synapsis as the conjugation of homologous maternal and paternal chromosomes, we shall have at the conclusion of synapsis a reduced number of 18. That is, nine pairs or nine bivalant chromosomes and nine univalents which had been unable to find mates.

Such eggs, if they were fertilized by a spermatozoan containing 18 chromosomes should give rise to individuals with a somatic number of 36, or, uniting with a spermatozoan with 9 chromosomes should retain a somatic number of 27.

If the theory of the individuality of the chromosomes is correct and the interpretation of synapsis well founded, experiments in hybridization with favorable forms ought to prove the truth of such an explanation. The starfish, owing to our inability to raise adults from the egg and to the extremely small size of its chromosomes, does not seem promising for such an investigation.

Summary.

1. The reduced number of chromosomes in the male germ cells of *Asterias vulgaris* is 9.

2. Fertilized starfish eggs contain as a somatic number 18 and 36 chromosomes, the difference possibly to be correlated with *Asterias vulgaris* and *Asterias forbesii*.

3. Eggs caused to develop parthenogenetically show one half the somatic number of chromosomes.

4. No conjugation of individual chromosomes takes place in fertilized eggs immediately before the first segmentation.

5. A possibly hybrid form contains 27 chromosomes.

Bryn Mawr College,
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All of the figures are from camera drawings made with aid of Zeiss No. 12 compensating ocular and 2 mm. apochromatic objective. Some of the sketches were subsequently doubled in diameter by means of a drawing camera. These have been reduced one half in reproduction. The others are reproduced as drawn.